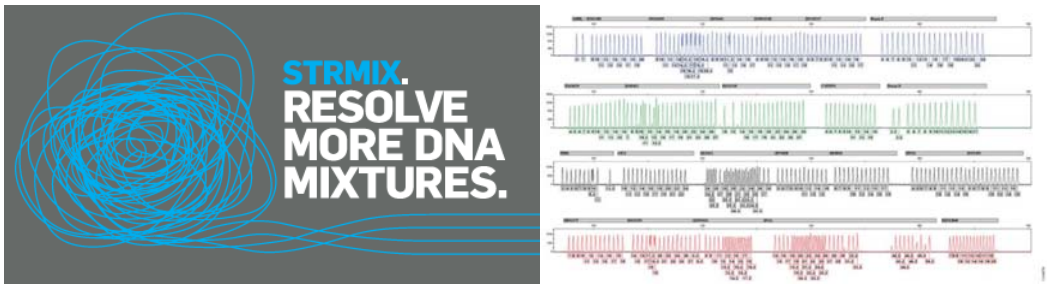
Attachment 14

Michigan State Police,
STRMix Validation Summary

Michigan State Police
Forensic Science Division

Validation Summary

STRMix-PowerPlex Fusion



Michigan State Police
Forensic Science Division

TABLE OF CONTENTS

The FBI Quality Assurance Standards for Forensic DNA Testing Laboratories (dated 09/01/2011) includes the following standards for internal validations.

Standard	Title
8.3.1.1	Known and Non-probative Evidence Samples or Mock Evidence Samples
8.3.1.2	Reproducibility and Precision
8.3.1.3	Sensitivity and Stochastic Studies
8.3.1.4	Mixture Studies
8.3.1.5	Contamination Assessment (N/A)

In addition to the standards listed above, the Scientific Working Group on DNA Analysis Methods (SWGDAM) published guidelines entitled SWGDAM Guidelines for the Validation of Probabilistic Genotyping Systems (dated 06/15/2015).

The standards and guidelines referenced above have been reviewed and provide the necessary documentation required by the FBI Director's "Quality Assurance Standards for Forensic DNA Testing Laboratories" for internal validation.

February 22, 2016

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Date

Purpose:

This work validates the use of the software application STRMix™ as a probabilistic genotyping tool to assist in the interpretation of DNA testing profiles from Promega's PowerPlex® Fusion STR amplification chemistry at 30 cycles using an Applied Biosystems 3500/3500XL capillary electrophoresis instrument.

Background Information:

Forensic DNA testing began in the mid-1990s in Michigan with the utilization of a methodology called Restriction Fragment Length Polymorphisms (RFLP). This technique required a considerable amount of DNA (approximately 500 ng) from crime scene samples to obtain a result to be used for comparison to DNA samples from potential donors. In 1998, the Michigan State Police migrated to a more sensitive and efficient laboratory method for DNA testing called Short Tandem Repeats (STRs) utilizing capillary electrophoresis instruments. At that time, STRs testing required approximately 1-2 ng of DNA to obtain a profile with a genetic analyzer run time of roughly 30 minutes per sample. This technology was also better suited for mixed DNA samples of more than one contributor and samples that were degraded.

Since the late 1990s, the Michigan State Police has continued with the STRs technology, but has adopted improved STR amplification chemistries and genetic analyzers that have incorporated more genetic markers, shorter amplification times, increased sensitivity (500 pg), smaller DNA target regions, improved response to inhibitors, increased genetic analyzer capacity (24 samples per 45 minutes) and many other factors. With this improved technology and capacity, a significantly broader range of evidentiary samples have become eligible for testing in the DNA laboratory. However, with the broader range of samples submitted and increases in overall sensitivity, the DNA laboratories have seen a significant increase in the number of DNA samples with multiple contributors, artifacts, stochastic events, allelic drop in/out and other factors that must be considered during DNA profile interpretation.

Many forensic DNA laboratories in the United States are currently evaluating software applications to assist with the interpretation of DNA profiles. One such application created by the Institute of Environmental Science and Research (ESR), located in New Zealand, is termed

STRMix™. It is described as a fully continuous DNA profile interpretation application that may be used to standardize DNA analysis within the laboratory. It uses a probabilistic approach to profile interpretation using a laboratory's own DNA data to estimate the variance anticipated in the DNA electropherogram data. The developers have completed the necessary developmental validation studies and the modeling and mathematical basis for STRMix™ have been published in numerous professional journals that undergo peer review. The results of the developmental validation can be viewed in the 2.3 User's Manual.

The Michigan State Police has evaluated the STRMix™ application version 2.3.07 for use at our Northville, Lansing and Grand Rapids DNA Laboratories. Manufacturer guidelines were followed and can be viewed in the 2.3 Implementation and Validation Guide, along with the formulas and spreadsheets.

STRMix™ is able to use data from the Michigan State Police DNA laboratories to develop parameters utilized within the application by the use of the Model Maker application found within the broader STRMix™ application. Specifically, these parameters include:

- Analytical Threshold (AT) sometimes referred to as the Peak Amplitude Threshold (PAT)
- Capillary Electrophoresis camera saturation
- Stutter ratios
- Drop-in parameters
- Locus specific amplification efficiency parameters
- Peak height variance
- Hyper-parameter for the variance of locus specific amplification effects
- Allele and stutter peak height variance prior distributions

STRMix™ cannot assist with the determination of the number of contributors to any given DNA profile. This must be entered by the user at the time of analysis. Additionally, the profile data must be provided to the STRMix™ application before analysis. The profile data is provided typically as a .txt file generated from GeneMapper IDx following evaluation by a trained DNA expert. Because STRMix™ provides a statistical estimate termed a likelihood ratio, the user will be required to establish the H_1 (prosecution) and H_2 (defense) hypotheses within the application prior to analysis.

This validation of the STRMix™ software application specifically addresses the forensic DNA testing technology currently in use at the Michigan State Police Forensic Science Division. This includes, in part, Promega's PowerPlex® Fusion STR amplification chemistry, Applied Biosystems 3500/3500xl Genetic Analyzers and GeneMapper® IDx software. The STR amplification chemistry was previously validated at 30 amplification cycles. The Applied Biosystems genetic analyzers were previously validated to establish injection, run parameters and an analytical threshold (250 RFUs). Most run parameters are pre-determined, except for the injection times. An initial injection time of 18 seconds is utilized, with the ability to increase the injection time to 28 seconds or reduce the injection time to 10 seconds dependent upon the quality of the DNA profile obtained.

STRMix™ Parameter Establishment:

The first step of the validation of STRMix™ involved collecting DNA data from existing samples using the current procedures, guidelines, chemistries and equipment to establish the numerous parameters the application utilizes. The Michigan State Police Forensic Science Division is comprised of three DNA laboratory locations using the same analytical procedures and equipment. Even though each laboratory location utilizes the same analytical procedures and equipment, DNA data was collected and evaluated from each laboratory location to establish parameters specific to that location. Once the parameters for each location were determined, they were compared to determine if there was any significant difference between them. The settings described below are from one of the three laboratories and was determined to be most representative of the collective three laboratories.

Analytical Threshold (AT):

The assignment of a signal as allelic product as opposed to baseline or noise is important in DNA profile analysis. This differentiation is usually undertaken using a set threshold above which peaks are deemed to be allelic if they also meet certain morphological requirements, and below which they are ignored, regardless of morphology. The issue is to assign a threshold, often termed the limit of detection (LOD) or analytical threshold (AT), to minimize the detection of artifacts while maximizing the detection of allelic peaks.

Optimum AT values have previously been determined by the Michigan State Police Forensic Science Division for all the Fusion loci and a kit specific threshold had been set at 275 RFUs. This value was modified slightly to a lower analytical threshold of 250 RFUs and was used for all data analysis within this report.

Stutter:

There are three parameters within STRmix™ that calculate expected stutter rates and therefore require optimization. The first is the maximum allowable stutter ratio. The maximum allowable stutter ratio reduces run time by only permitting peaks in a stutter position below a certain percentage to be considered stutter. This parameter has been set at 0.3 (30%) based on inspection of laboratory stutter ratio data.

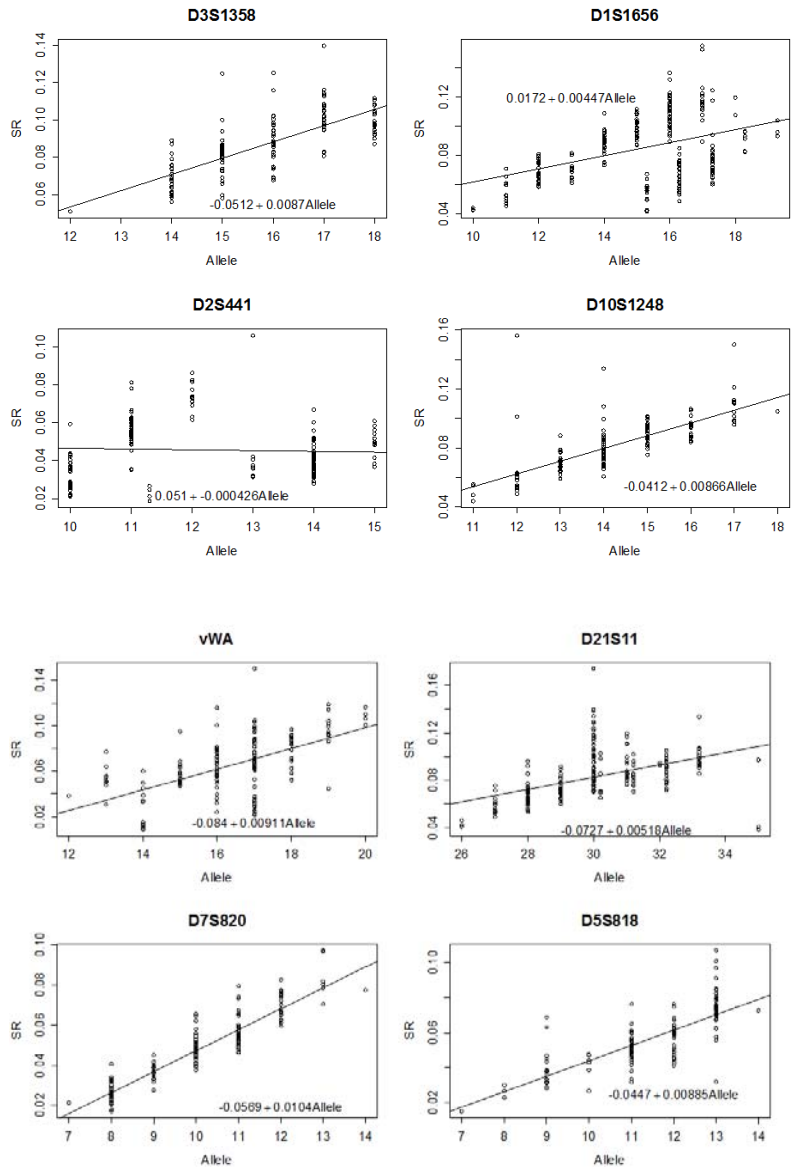
The second parameter is a file used to model the expected heights of the stutter peaks based on their partner allele designation. The values used to determine expected stutter heights are 'per allele'. Per allele stutter ratios are calculated using a linear equation and regressing stutter ratio against allele. Within STRmix™, stutter is estimated using the model **Error! Objects cannot be created from editing field codes.** where the intercept (*c*) and slope (*m*) are determined using regression. Values for *m* and *c* were previously calculated. A summary of the STRmix™ allelic stutter files is given in Table 1.

Table 1: Per allele Fusion stutter values for STRmix™

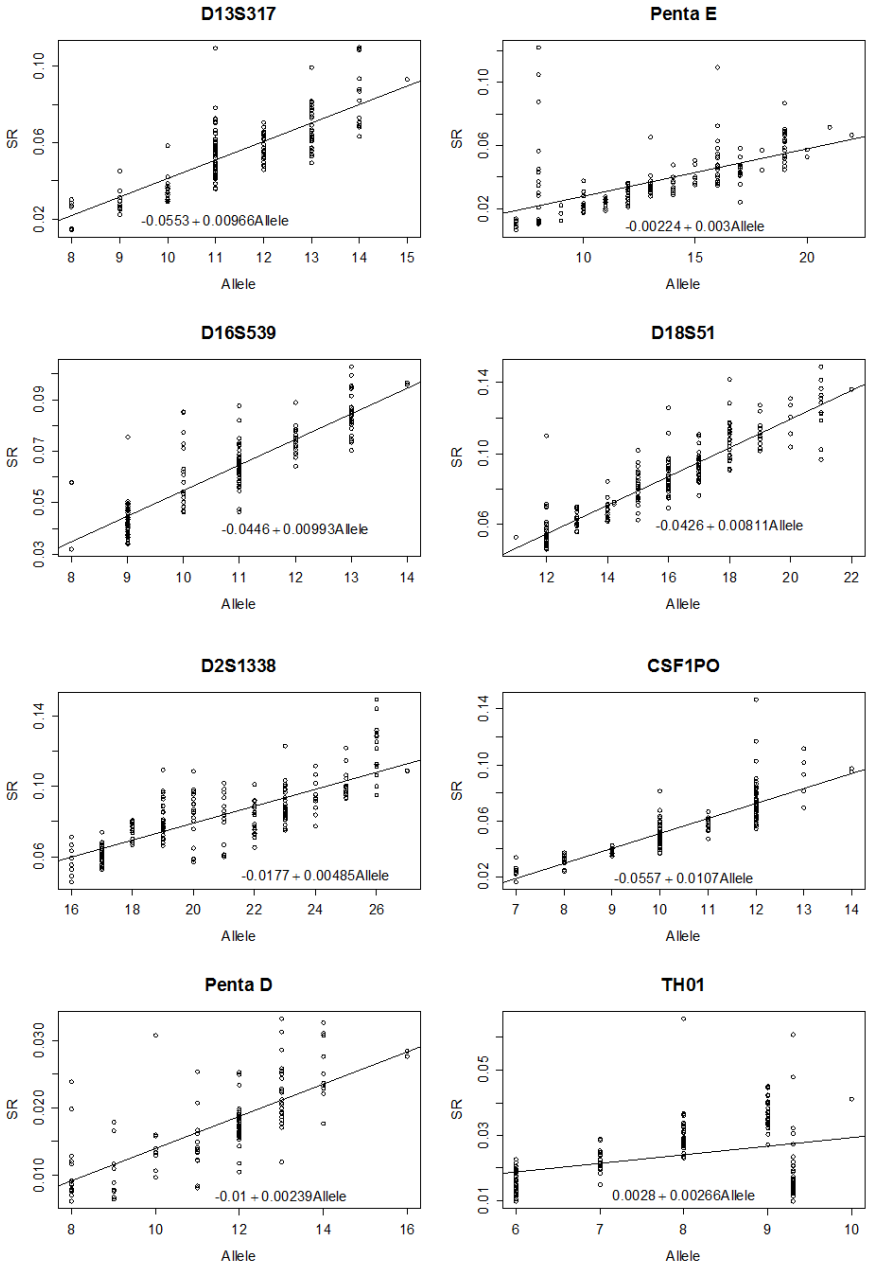
Marker	Intercept	Slope
D3S1358	-0.0512	0.0087
D1S1656	0.0172	0.00447
D2S441	0.051	-0.00043
D10S1248	-0.0412	0.00866
D13S317	-0.0553	0.00966
Penta E	-0.00224	0.003
D16S539	-0.0446	0.00993
D18S51	-0.0426	0.00811
D2S1338	-0.0177	0.00485
CSF1PO	-0.0557	0.0107
Penta D	-0.01	0.00239
TH01	0.0028	0.00266
vWA	-0.084	0.00911
D21S11	-0.0727	0.00518
D7S820	-0.0569	0.0104
D5S818	-0.0447	0.00885
TPOX	-0.0379	0.00692
DYS391	NA	NA

Michigan State Police
Forensic Science Division

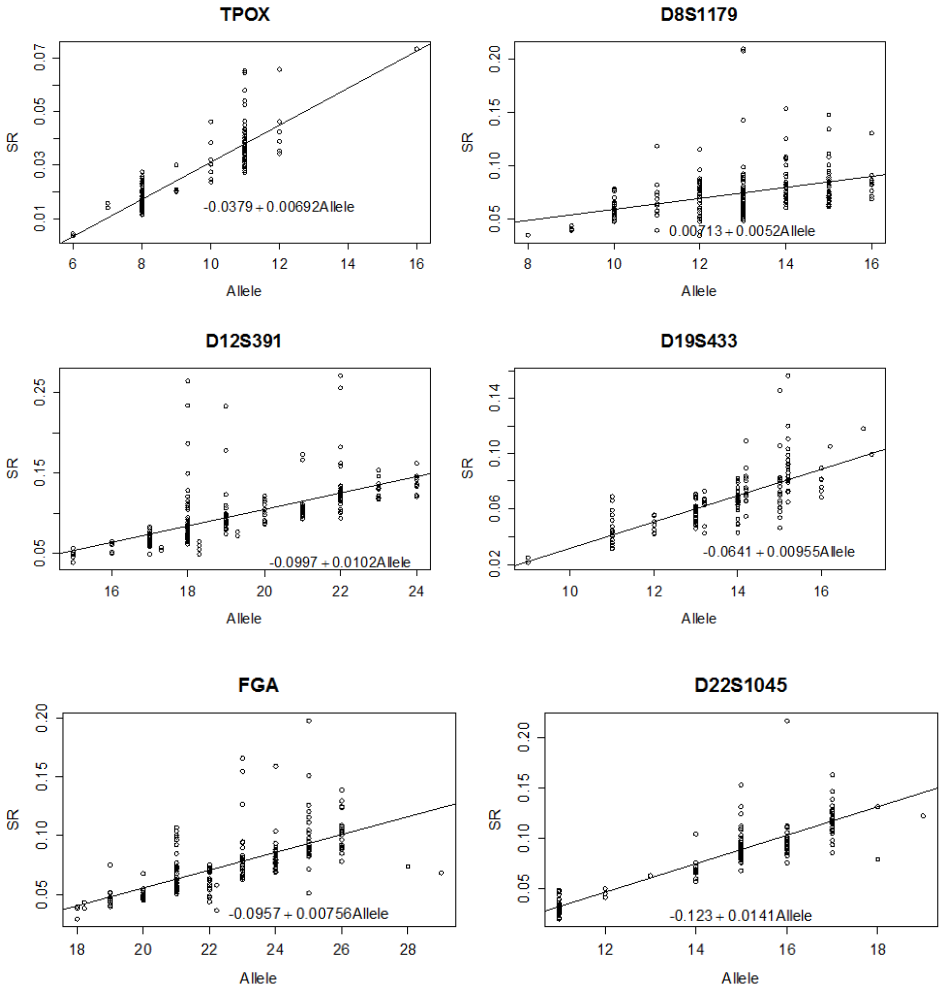
D8S1179	0.00713	0.0052
D12S391	-0.0997	0.0102
D19S433	-0.0641	0.00955
FGA	-0.0957	0.00756
D22S1045	-0.123	0.0141



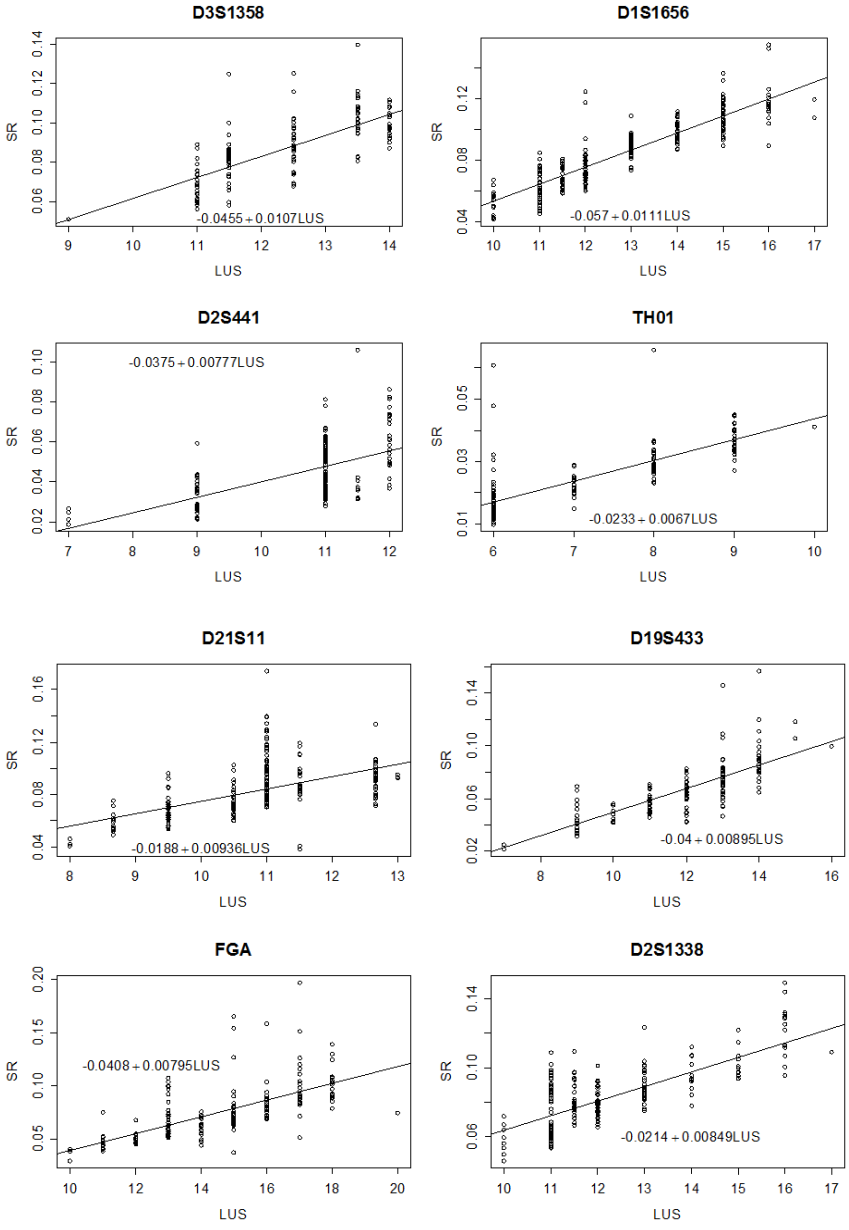
Michigan State Police
Forensic Science Division



Michigan State Police
Forensic Science Division



A better explanatory variable for a stutter ratio for loci with compound and complex structure has been shown to be the longest uninterrupted stretch of common repeats (LUS) within the allele and not the allele designation itself. Values for LUS are determined by sequencing alleles. A number of common alleles for forensic loci have been typed. A summary of these appear on STRBase. A plot of *SR* versus LUS for compound and complex loci within the PowerPlex® Fusion multiplex is provided here.



The third parameter within STRmix™ that determines expected stutter peak heights is an exception file based on either LUS or an average observed stutter ratio. LUS is used where it is a good explanatory variable for *SR* otherwise the average of the observed *SR* is used. A stutter exception file based on laboratory data has been created and was used in this analysis. Where

alleles are not present in this file the expected stutter rates are calculated from the allele file (Table 1).

Drop-in parameters:

Drop-in is non-reproducible, unexplained peaks observed within a profile. There are four parameters used for the modelling of drop-in in STRmix™. These are:

1. Z: the detection threshold or analytical threshold
2. A cap on the maximum allowed combined drop-in height per locus
3. The drop-in frequency
4. α,β: two parameters for the gamma model.

Drop-in rates for a laboratory platform (multiplex and instrument combination) should be monitored. This is done by recording counts and corresponding heights of drop-in peaks observed in negative controls and counts of negative controls without drop-in peaks. Within STRmix™ drop-in is modelled using a gamma distribution.

The drop-in parameters were determined as described in the STRmix™ Implementation and Validation Guide and the optimized parameters are provided in Table 2.

Table 2: Drop-in parameters for STRmix™ for the Fusion data

Drop-in cap	400
Drop-in frequency	0.3453
Drop-in parameters	0.06,95.52

Saturation:

The peaks in a DNA profile are measured using fluorescence. The amount of fluorescence is proportional to the amount of DNA present. This fluorescence is captured by a camera. It is expected that as more DNA is added into a PCR the resulting peak height (measured in relative fluorescent units) in an electropherogram will increase. The camera can become saturated when there is too much fluorescence detected. This means we can no longer accurately measure the

height of the peaks observed or estimate how much DNA is really represented by this result. Following this we can no longer accurately model over saturated peak heights using STRmix™.

The saturation setting is the upper limit for a peak's height permitted in the software, beyond which the model is no longer optimal. The software will treat peaks in the input evidence data above this value as qualitative only. Saturation, like the analytical threshold, is mostly instrument related and not kit or method dependent.

The expected height of every allele within the stutter ratio dataset was calculated using the formula:

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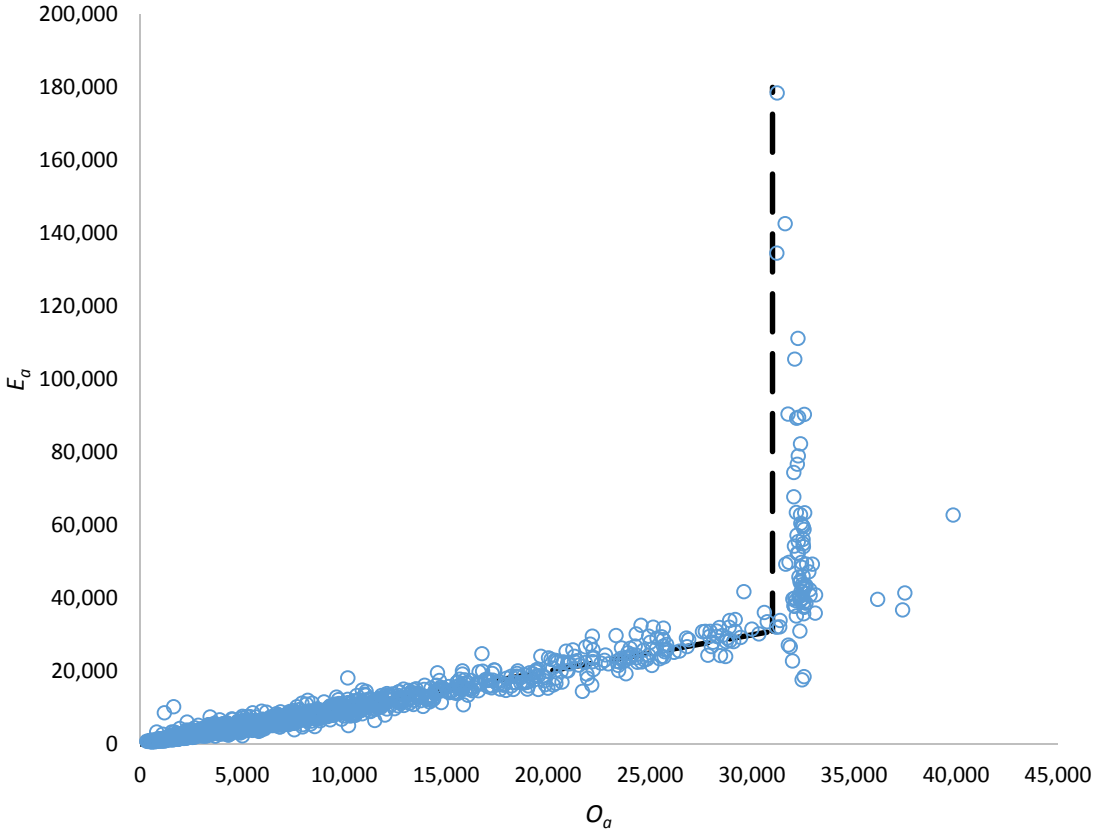
Where (E_a) is the expected peak height calculated from the observed stutter height (O_{a-1}) and **Error! Objects cannot be created from editing field codes.** is the expected stutter ratio for allele *a* calculated using the values for simple repeats from Table 1. A plot of E_a versus O_a is provided in Figure 1. A vertical line at O_a = 31,000 rfu indicates the saturation limit for this dataset. The points should deviate from the $x = y$ line at the saturation value. After inspection of Figure 1 we recommend a saturation threshold setting of 31,000 rfu is applied.

Figure 1: Observed versus expected peak heights



Peak height variance and LSAE using Model Maker:

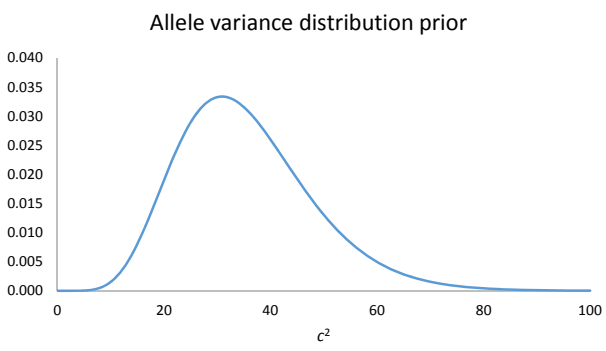
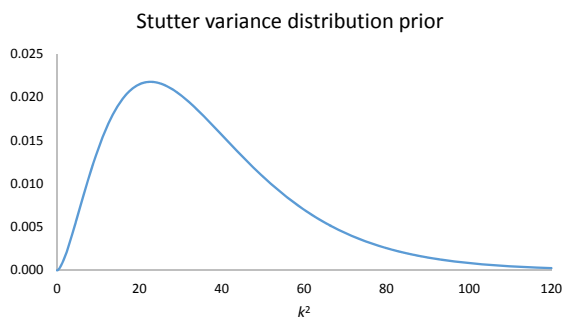
Empirical observations and experience suggests that profiles differ in variance (hereafter "quality"). Within STRmix™ the variability of peaks within profiles is described using a model containing a variance constant. Within V2.3 allele and stutter peaks have separate variances, c^2 and k^2, respectively. The c^2 and k^2 terms are variables which are determined after sampling from a gamma distribution within the MCMC.

140 single source profiles of varying quality were analysed using the Model Maker function within STRmix™. A summary of the results for both c^2 and k^2 for the dataset is provided in Table 3. A plot of the allele and stutter gamma distributions are provided in Figure 2.

Table 3: Summary of Model Maker results for the dataset

Multiplex	Number profiles analysed	Allele variance parameters (Mode)	Stutter variance parameters (Mode)	Mean LSAE variance
Fusion 5C	140	7.853, 4.508 (30.896)	2.690, 13.425 (22.694)	0.0297

Figure 2: A plot of the allele and stutter gamma distributions for each dataset

Allele	Stutter
	

Heterozygote balance was calculated for all heterozygote loci for the Model Maker profiles. Heterozygote balance (*Hb*) was calculated as:

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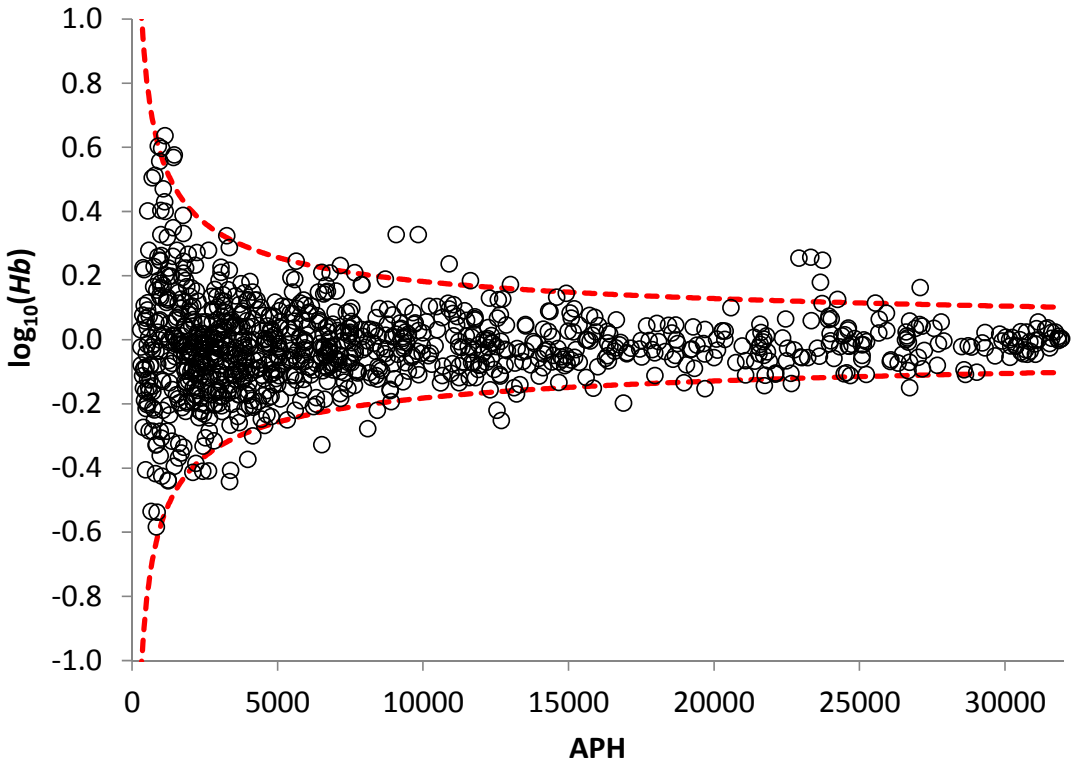
Where **Error! Objects cannot be created from editing field codes.** refers to the observed height of the high molecular weight allele and **Error! Objects cannot be created from editing field codes.** the observed height of the low molecular weight allele. Previous work has suggested that there is a relationship between the variation in peak height and the variation in *Hb* [6, 7]. In single source profiles, variability in *Hb* reduces as the average peak height (APH) at a locus increases. The variance of *Hb* is expected to be twice the variance of the individual allelic peaks assuming the variance of each peak is the same. This allows an approximate comparison between the variance from the STRmix™ MCMC approach and a readily determined variable from empirical data.

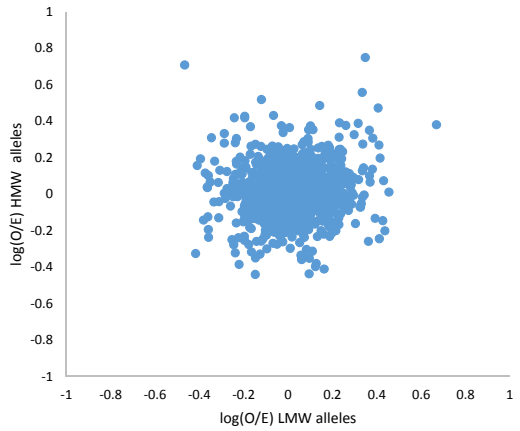
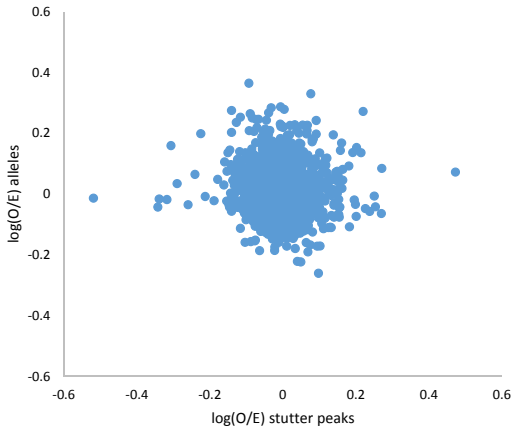
The plot of log*Hb* versus APH for each of the datasets described above and the expected 95% bounds (plotted as dotted lines) calculated at **Error! Objects cannot be created from editing field codes.** where**Error! Objects cannot be created from editing field codes.**= 43, the 75th percentile from the gamma distribution from the data set. The 95% bounds encapsulate sufficient data as demonstrated in the graphs (coverage = 95.8%) demonstrating that the values for variance are sufficiently optimised. The plot in Figure 3 is an approximate check of Model Maker.

Figure 3: Log(*Hb*) versus APH for single source profiles



In Figure 4 we plot the correlation plots for LMW versus HMW allele and allele versus stutter peaks for the Model Maker dataset. The distribution of the points within the figures is as expected, with no observed correlation. There are some outliers observed in the logarithm of the observed over expected stutter peak height versus log(O/E) allelic peak height plot. These are larger than expected stutter peaks that were labelled at analysis however they do not affect the results.

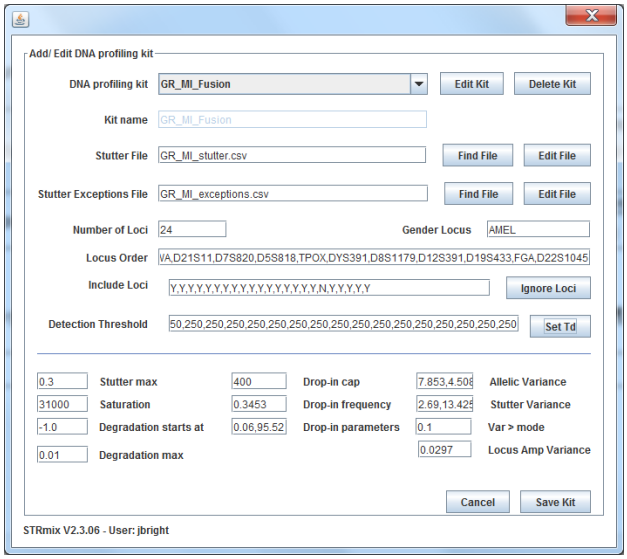
Figure 4: PowerPlex Fusion correlation plots

HMW and LMW alleles, correlation = 0.064	Allele and stutter, correlation = -0.052
	

Default Parameters Conclusion:

The recommended STRmix™ V2.3 default parameters for the interpretation of the Powerplex® Fusion 5C profiles run on a 3500 CE instrument are given in Figure 5.

Figure 5: STRmix™ recommended default parameters for PowerPlex® Fusion profile interpretation



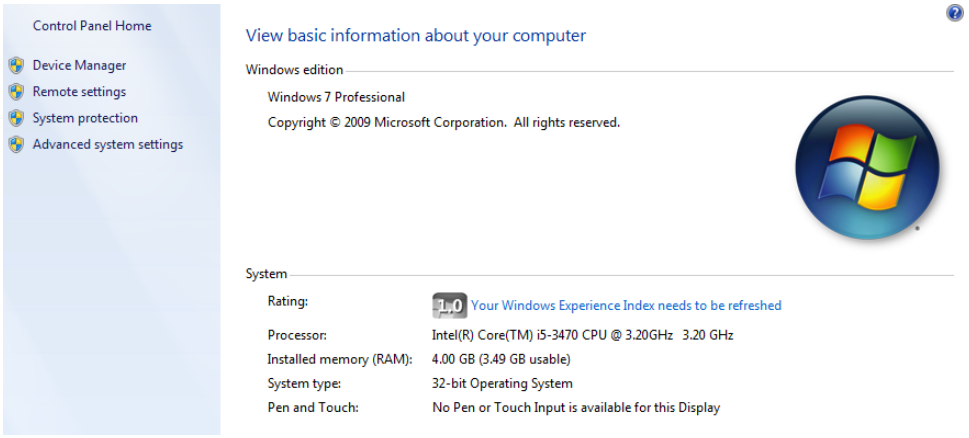
Internal Validation of STRMix™ with Established Parameters:

The following internal validation studies are organized to follow the guidelines as outlined in: *SWGDAM Guidelines for the Validation of Probabilistic Genotyping Systems* (as published).

2.1 The laboratory should verify that the software is installed on computers suited to run the software, that the system has been properly installed, and that the configurations are correct.

At the time of this validation summary, the STRMix™ licenses are installed on computers that also serve as GeneMapper® IDx data processing terminals. The computers are identical and have the following information:

Figure 6: STRMix™ Computer Specifications



The STRMix™ v2.3 Installation Manual system requirements have the following minimum specifications:

- Intel Core 2 (Quad Core) processor
- 4GB RAM
- 10MB free HDD space
- Windows XP Professional

The following are additional recommended specifications:

- Intel Corei5
- i7 processor or better

- 16GB RAM or more
- 10MB free HDD space
- Windows 7 Professional 64 bit.

A review of the specifications of the Forensic Science Division GeneMapper® IDx computers indicates they exceed the minimum requirements for STRMix™ operation.

2.2.1 Every software release should have a unique version number. This version number should be referenced in any validation documentation or published results.

This validation summary and each of the individual studies were completed using the STRMix™ application version 2.3.07. Future versions of the software may require either an additional validation or performance verification, depending on the extent of differences between the validated and newer versions

2.2.2 Appropriate security protection to ensure only authorized users can access the software and data.

Each computer with an installed version of STRMix™ will be located within the Biology/DNA work area with limited security access to staff members assigned to that specific area. Additionally, the computer is further secured with a password only available to authorized individuals. Data developed during a STRMix™ analysis will only be housed on the computer in which it is generated or uploaded to the case file within the Forensic Advantage laboratory information management system.

2.2.3 Audit trails to track changes to system data and/or verification of system settings in place each time a calculation is run.

The STRMix™ application relies upon a number of different settings typically established during the internal validation. The settings include, but are not limited to, stutter ratios, gamma distribution parameters, drop-in rates, saturation levels etc. The STRMix™ output, including the standard and advanced reports, include a section with a summary of the settings used for that particular STRMix™ calculation. Once written, the Michigan State Police analytical procedures will include a verification of the settings used to the established and validate settings to ensure the correct ones were utilized.

2.2.4 User-level security to ensure that system users only performed authorized actions.

STRMix™ does not have features that can be specifically assigned to different user levels. For instance, STRMix™ does not have an administrator feature to set the specific analysis parameters within the software. For that reason, the DNA Procedure manual will require verification that the proper settings and parameters were utilized during case technical reviews.

4.1 The laboratory should test the system using representative data generated in-house with the amplification kit, detection instrumentation and analysis software for casework. Additionally, some studies may be conducted by using artificially created or altered input files to further assess the capabilities and limitations being evaluated.

All data used for the internal validation of STRMix™ was generated within the Michigan State Police Forensic Science Division laboratories. Each of the three DNA laboratories (Lansing, Northville and Grand Rapids) generated data utilized in the Model Maker module to establish internal parameters for STRMix™. The parameters from the Grand Rapids laboratory from the Model Maker analysis provided the most conservative values as compared to those generated from data provided by the Lansing and Northville laboratories. The internal validation utilized the Grand Rapids values. The majority of the case data was generated at the Lansing Laboratory utilizing standard procedures and equipment including Plexor® HY, PowerPlex® Fusion (30 cycles), 3500/3500XL capillary electrophoresis and GeneMapper® IDx data analysis software. Additionally, STRMix™ analyses covered the range and scope of profiles typically encountered in the laboratory. These included various contributor numbers (up to and including four contributors), the range of input amplification DNA amounts, and genetic analyzer run and injection conditions (10, 18 and 28 second injections). Adjudicated case analysis using STRMix™ was completed at each of the three Michigan State Police Forensic Science Division DNA laboratories.

DNA profiles for most studies were unaltered from the data generated in the laboratory. However, some studies required *in silica* alterations to the DNA data to simulate rare instances that are difficult to generate in the laboratory setting. These studies included instances of drop-in, drop-out and degraded profiles.

4.1.1 Specimens with known contributors, as well as case-type specimens that may include unknown contributors.

This section will address the unknown contributors using the case-type specimen guideline as detailed under 4.1.1. The known contributor profiles that were evaluated with STRMix™ are further detailed under section 4.1.6 Mixed Specimens.

Approval was obtained to further evaluate adjudicated cases that were previously analyzed using the PowerPlex® Fusion STR amplification chemistry with the STRMix™ software application. To date, more than 55 items from adjudicated cases have been analyzed through STRMix™. These items were randomly selected to best represent situations whereby the initial interpretation either included an individual, excluded an individual or the overall interpretation of the item was inconclusive. The following table summarizes the findings from the initial interpretation and the STRMix™ interpretation.

Table 4: Adjudicated Cases

Item Description	Initial Interpretation	Statistical Value Reported	STRMix Results
bs from boxer shorts	Mixed sample too complex	NA	Inclusion 6Ax 5.88E9, run as 2 cont
swabs Mt Dew can	2 donors, exclusion 3Ax & 6Ax from the major donor, minors not suitable for comparisons	NA	NA still no conclusions for the minor donor
swabs of rifle	2 donors major male donor match 3Ax to major donor	1.2 Septillion	Inclusion 3Ax 4.52E24
swabs zip-tie inside surfaces	2 donors major male donor match 2Ax minors not suitable	1.3 Octillion	Inclusion 2Ax 5.05E24, still no conclusions for the minor
swabs inside handcuffs	2 donors inconclusive	NA	8.21E20, 2Ax could now be included
steering wheel swabs	at least2 donors inconclusive	NA	6.75E0 3Ax inconclusive
Crush Grape Bottle	2 donors, CPI 3Ax, 6Ax excluded	429.7 Billion	3.78E26 3Ax still included, 6Ax still excluded
outside condom epi	at least 2 donors, major matches 4Ax, 3Ax included as a minor	RM = 1.1 Octillion, CPI = 382.6 Billion	3Ax = 3.25E20, 3Ax & 4Ax included = 3.14E47

Michigan State Police
Forensic Science Division

driver's side inside door handles	Mixed sample too complex	NA	6Ax included = 1.31E3, 3Ax excluded,
bs from boxer shorts	Mixed sample too complex	NA	Inclusion 6Ax 1.60E16 run as 3 cont, 5Ax excluded.
bs from boxer shorts	Mixed sample too complex	NA	Inclusion 6Ax 2.10E24, exclude 5Ax
bs from boxer shorts	Mixed sample too complex	NA	Inclusion 6Ax 7.34E28, exclusion 5Ax
swabs of inside of handcuffs	Mixed sample too complex	NA	2Ax excluded
knife blade	2 donors with major female, L10x matched to major donor, minor not suitable	107.4 octillion	L10x included = 1.89E26; minor still not suitable
swabs of knife handle	2 donor with major male, 3x matched to major donor, L10x excluded, minor not suitable	18.6 octillion	3x included = 3.39E26; L10x included = 3.23E12; run with both knowns = 7.31E39
handle of vice grips	2 donors with major male, 3x matched to major donor, minor not suitable	18.6 octillion	
front interior panel of panty - sperm	no conclusions due to limited data	NA	run as 2: Px included = 8.11E10, exclusion ZZx
interior crotch panel and sides of crotch panel - sperm	at least 3 donors, intimate sample with vic included (2Ax), Px match to male donor, ZZx excluded	49.3 quadrillion	condition on vic: Px included = 1.38E15
Mossberg shotgun	Mixed sample too complex	NA	NA
bs on arms of Taren Walters	3 donors including 1x major matches 2Ax, no conclusions minor	351.9 Quadrillion	Inclusion 2Ax 2.55E18, 2Bx (minor) inconclusive 1.36E2
bs finger of Taren Walters	3 donors including 1x, major matches 2Ax, no conclusions minor	2.0 Quintillion	Inclusion 2Ax 2.30E20, 2Bx excluded
bs tennis shoe	2 donors, major matches 2Ax, no conclusions minor	264.9 Quintillion	Inclusion 2Ax 3.18E19, 1x included 1.36E5
bs tennis shoe			not compared
swabs of gear shifter	Mixed sample too complex	NA	Inclusion 3Ax 3.83E3, 6Ax 2.24E6
swabs of gear shifter	Mixed sample too complex	NA	Inclusion 6Ax 1.80E4 & 3Ax 2.21E2 inconclusive

Michigan State Police
Forensic Science Division

bs from buttoned shirt	2 donors, 6Ax matches major donor, minor not suitable	268.9 Decillion	Inclusion 6Ax7.54E33, 5Ax excluded
Pass side rear inside door handles	Mixed sample too complex	NA	Exclusion 6Ax
steering wheel swabs	at least 2 donors inconclusive	NA	4.18E2 3Ax inconclusive, 3.41E8 6Ax could now be included
black and grey scarf	at least 3 donors, Ax matches major donor, additional donors not suitable	1.2 million	Ax included = 5.28E18
black and grey scarf	at least 3 donors, Ax matches major donor, additional donors not suitable	1.2 million	Ax included = 3.63E18
black scarf	Mixed sample too complex	NA	Ax excluded = 1.19E-4
.380 caliber cartridges	Mixed sample too complex, no knowns for comparison	NA	Partial major donor
shotgun trigger	partial mixture of 2 ppl, major male; 2Ax excluded	NA	2Ax excluded = 1.32E-6
shotgun fore-end & stock	2 donors, major male; 2Ax excluded	NA	2Ax excluded
Swabs - interior cuffs jacket	(Buccal swab) matches the major donor to item (Swabs-interior cuffs of jacket). IDENTITY		1.48E+26
Swabs-interior neckline of jacket	A mixed DNA profile was obtained from item (Swabs-interior neckline of jacket). Due to the complexity of the profile, no conclusions can be made.		Not ran with STRmix - too complex
Swabs of rear passenger door handle L-1	Items (Buccal swab) and (DNA bloodstain card) are excluded as major donors to item (Swabs of rear passenger door handle L-1).		1.62E+08
Swabs of rear passenger door handle L-1	Items (Buccal swab) and (DNA bloodstain card) are excluded as major donors to item (Swabs of rear passenger door handle L-1).		8.48E-07
Swab of left hand fingernail clippings	Items (Buccal swab) and (DNA bloodstain card) are excluded as major donors to item (Swabs of front passenger door handle L-2).		2.90E+01
Swab of left hand fingernail clippings	Items (Buccal swab) and (DNA bloodstain card) are excluded as major donors to item (Swabs of front passenger door handle L-2).		1.11E+01

Swabs of pocket (rear) driver's seat	Item (Buccal swab) matches the major donor to item (Swabs of pocket (rear) driver's seat). (STATISTIC #1) - IDENTITY		7.53E+23
Swabs of pocket (rear) driver's seat	Item (DNA bloodstain card) is included as a possible contributor to item (Swabs of pocket (rear) driver's seat). (STATISTIC #2) CPI is 1 in 87.26 million Cauc		3.06E+14
Swabs of pocket (rear) driver's seat	Both present in mixture		7.44E+38
Swab from garage door opener	A partial DNA profile of two donors was obtained from item (Swab from garage door opener). Due to the limited data obtained, no conclusions can be made.		2.48E+08
Swab from garage door opener	A partial DNA profile of two donors was obtained from item (Swab from garage door opener). Due to the limited data obtained, no conclusions can be made.		2.40E+01
Swab from interior front driver door	Item (Buccal swab) matches the major donor to item (Swab from interior front driver door). IDENTITY		6.29E+28
Swab from interior front driver door	Item (Buccal swab), item (Buccal swab), and item (Buccal swab) are excluded as major donors to item (Swab from interior front driver door).		0.00E+00
Swab from interior front driver door	Item (Buccal swab), item (Buccal swab), and item (Buccal swab) are excluded as major donors to item (Swab from interior front driver door)		0.00E+00
Swab from interior front driver door	Item (Buccal swab), item (Buccal swab), and item (Buccal swab) are excluded as major donors to item (Swab from interior front driver door).		0.00E+00
Swab from interior front passenger door	A mixed DNA profile was obtained from item (Swab from interior front passenger door). Due to the complexity of the profile, no conclusions can be made.		Not ran with STRmix - too complex
Swab from steering wheel	Item (Buccal swab) matches the major donor to item (Swab from steering wheel). IDENTITY		1.67E+30

Swab from steering wheel	Items (Buccal swab), item (Buccal swab), and item (Buccal swab) are excluded as major donors to item (Swab from steering wheel).		2.50E-17
Swab from steering wheel	Items (Buccal swab), item (Buccal swab), and item (Buccal swab) are excluded as major donors to item (Swab from steering wheel).		0.00E+00
Swab from steering wheel	Items (Buccal swab), item (Buccal swab), and item (Buccal swab) are excluded as major donors to item (Swab from steering wheel).		0.00E+00
Swab from Colt .45	A mixed DNA profile was obtained from item (Swab from Colt .45). Due to the complexity of the profile, no conclusions can be made.		Not ran with STRmix - too complex
Swab from H&R .22	A mixed DNA profile was obtained from item (Swab from H&R .22). Due to the complexity of the profile, no conclusions can be made.		5.06E+04
Swab from H&R .22	A mixed DNA profile was obtained from item (Swab from H&R .22). Due to the complexity of the profile, no conclusions can be made.		5.19E-11
Swab from H&R .22	A mixed DNA profile was obtained from item (Swab from H&R .22). Due to the complexity of the profile, no conclusions can be made.		9.61E-34
Swab from H&R .22	A mixed DNA profile was obtained from item (Swab from H&R .22). Due to the complexity of the profile, no conclusions can be made.		2.93E+11
Swab from H&R .22	A mixed DNA profile was obtained from item (Swab from H&R .22). Due to the complexity of the profile, no conclusions can be made.		6.41E-16
Swabs of handgun	Item (Buccal swab) is included as a possible contributor to item (Swabs of handgun). CPI is 1 in 1,253 Caucasian		0.00E+00
Swabs of handgun	Item (Buccal swab) is included as a possible contributor to item (Swabs of handgun). CPI is 1 in 1,253 Caucasian		2.11E+00

.38 special cartridges	Item (#) matches the major donor to item (.38 special cartridges). IDENTITY		9.79E+32
.38 special #J043648	Item (#) matches the major donor to item (.38 special #J043648). IDENTITY		4.58E+29
revolver	A mixed DNA profile was obtained from item (revolver). Due to the complexity of the profile, no conclusions can be made.		0.00E+00
revolver	A mixed DNA profile was obtained from item (revolver). Due to the complexity of the profile, no conclusions can be made.		0.00E+00
revolver	A mixed DNA profile was obtained from item (revolver). Due to the complexity of the profile, no conclusions can be made.		1.67E+09
revolver	A mixed DNA profile was obtained from item (revolver). Due to the complexity of the profile, no conclusions can be made.		3.77E+28
revolver	A mixed DNA profile was obtained from item (revolver). Due to the complexity of the profile, no conclusions can be made.		0.00E+00
revolver	A mixed DNA profile was obtained from item (revolver). Due to the complexity of the profile, no conclusions can be made.		9.03E+41
revolver	A mixed DNA profile was obtained from item (revolver). Due to the complexity of the profile, no conclusions can be made.		8.18E+12
revolver	A mixed DNA profile was obtained from item (revolver). Due to the complexity of the profile, no conclusions can be made.		8.77E+32
S&W 9mm	The DNA types obtained from item (S&W 9mm) are consistent with a mixture of three or more donors, including at least one unknown male contributor. Due to the complexity of the profile, no comparisons can be made.		STRmix Error
S&W 9mm magazine & ammo	A mixed DNA profile was obtained from (S&W 9mm magazine & ammo). Due to the complexity of the profile, no conclusions can be made.		2.40E-03

Michigan State Police
Forensic Science Division

Swab of Brass Knuckles	Mixture of 3 or more individuals. Major matches suspect-ran as 2 person	IDENTITY	1.89E+29
Swab of Brass Knuckles	Mixture of 3 or more individuals. Major matches suspect-ran as 3 person	IDENTITY	3.71e+28
baggies	A mixed DNA profile was obtained from (baggies). Due to the complexity of the profile, no conclusions can be made.		1.95E-06
Swabs of breasts	No statement in report?		4.64E+19
Swabs of breasts	Item (#) matches the male donor to item (Swabs of breasts). IDENTITY		1.87E+19
Swabs of breasts	Item (#) matches the male donor to item (Swabs of breasts). IDENTITY		7.01E+43
Swabs of breasts	Item (#) matches the male donor to item (Swabs of breasts). IDENTITY		STRmix Error … D12
safe handle	Item (#) matches the major donor to item (safe handle). IDENTITY		7.26E+26
lock box	Item (#) matches the major donor to item (lock box). IDENTITY		1.43E+27
"Premier" cigarette butt	A mixed DNA profile was obtained from item ("Premier" cigarette butt (DNA Extract)). Due to the complexity, no conclusions can be made.		5.79E+18
"Premier" cigarette butt	A mixed DNA profile was obtained from item ("Premier" cigarette butt (DNA Extract)). Due to the complexity of the profile, no conclusions can be made.		0.00E+00
"Premier" cigarette butt	A mixed DNA profile was obtained from item ("Premier" cigarette butt (DNA Extract)). Due to the complexity of the profile, no conclusions can be made.		0.00E+00
Hair root from hand	A mixed DNA profile from 2 individuals with a major/minor	Major match/one included/one inconclusive	Major 9.64E+40 Included and inconclusive are excluded (depending on hypotheses, inconclusives are included)
Hammer Head	A mixed DNA profile from 2 individuals.	Major match. Minor inconclusive	7.01E+27 Major Minor included

Michigan State Police
Forensic Science Division

Hammer Handle	A mixed DNA profile from 3 individuals	3 people included	8.89e+49 hypothesis of all 3 9.28E+22 hypothesis of 2
B/S outside condom	Major/Minor		2.49E+54 hypothesis includes both donors
Steering Wheel	A mixed profile from 3 individuals with a major donor	Major match, minor inconclusive	6.96E+30 hypothesis includes two donors
Pipe	A mixed profile with at least 4 donors	Too complex	4.79E20 hypothesis includes two donors Exclusion also reported for minor donor
Swab bbgun barrel	A mixed profile of 2 donors	Major match Minor inconclusive	5.96E+15 to major
Swab .45 caliber handgun	A mixed profile of 3 donors	Too complex	Too complex
Swab .38 caliber handgun	A mixed profile of 3 donors	Too complex	Exclusions
Steering wheel swabs	A mixed profile of 2 donors	Major match, minor inconclusive	4.85E+28 hypothesis for both donors
Blue Jeans	A mixed profile of 3 donors	Major match, minor inconclusive, person excluded	342 for minor exclusion 3.78E+31 for major match
Swab of bbgun	A mixed profile of 2 donors	Major match Minor Inconclusive	5.96E+15
Swab of bank card	A mixed profile of 2 donors	Exclusion	Exclusion
Shorts	A mixed profile of 2 donors	Match major and minor	1.15E+43 for both donors
Neck Swabs	A mixed profile of 3 donors	Victim major/inconclusive minor	7.62E+29 to victim

Labia Majora	A mixed profile of victim plus another donor	Major matches elimination Minor matches victim	2.53E+49

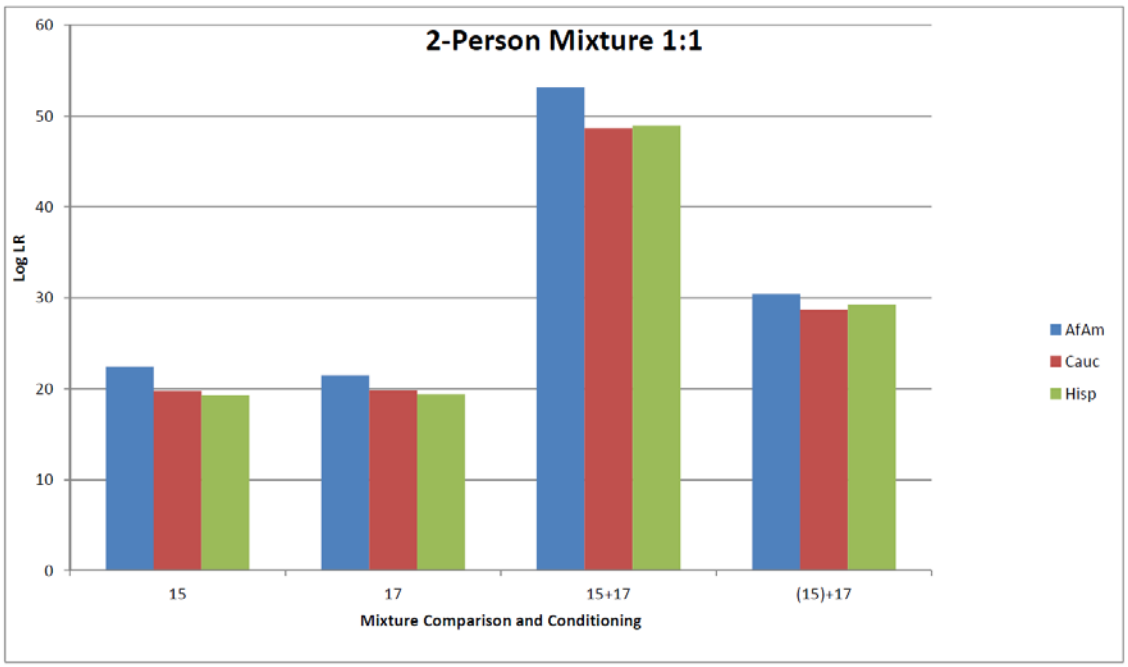
4.1.2 Hypothesis testing with contributors and non-contributors

4.1.2.1 The laboratory should evaluate more than one set of hypotheses for individual evidentiary profiles to aid in the development of policies regarding the formulation of hypotheses.

DNA reference samples were extracted using standard organic extraction methods and purified using Vivacon® centrifugal collectors. The concentration of the DNA samples was evaluated using Plexor® HY quantitation chemistry and an Applied Biosystems 7500 Real Time PCR instrument. DNA mixtures were prepared in the laboratory from the DNA reference samples. Once prepared, the DNA extracts for these mixtures were amplified using standard MSP procedures including the PowerPlex® Fusion STR amplification chemistry at 30 cycles and separated and detected using the Applied Biosystems 3500 Genetic Analyzer. Resulting electropherograms were evaluated using GeneMapper® IDx and exported to STRMix™ via the use of a .txt table file.

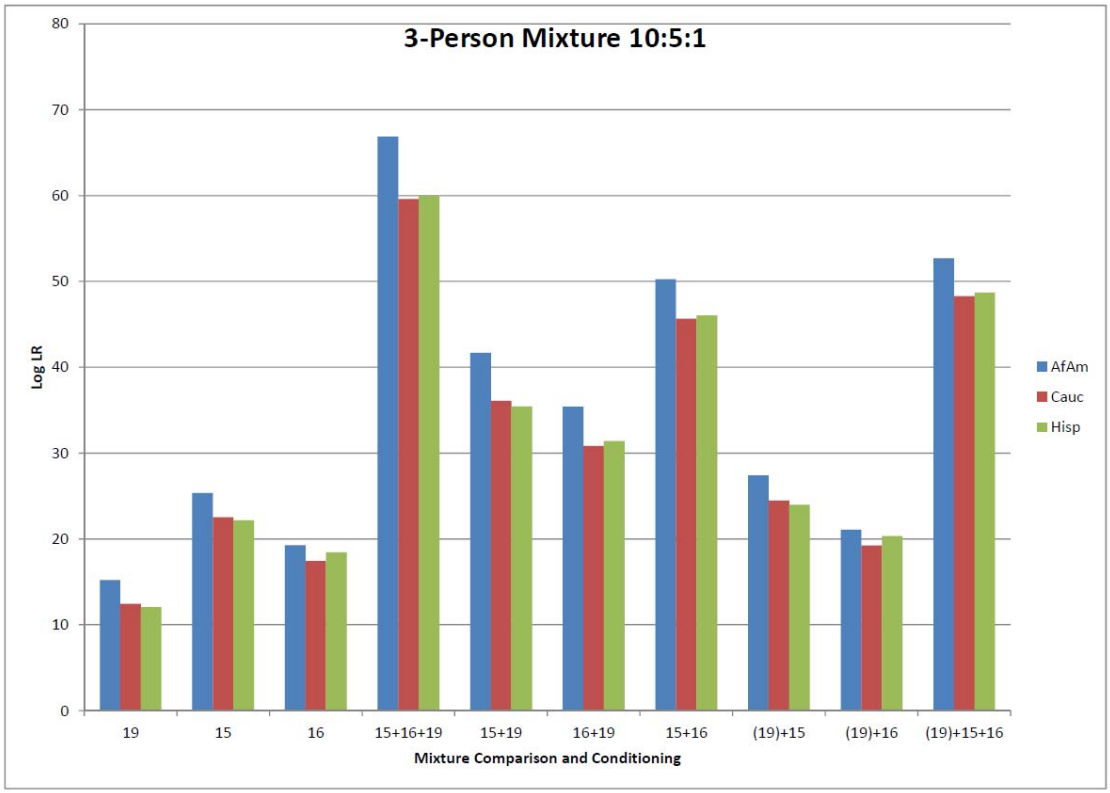
The 2-person mixture was a 1:1 mixture of each contributor. This ratio represents one of the more difficult samples for STRMix™ to deconvolute. Once deconvoluted, likelihood ratios were calculated for various conditions. For this particular mixture, contributor #15 was evaluated in the H_1 hypothesis. This scenario provided log likelihood ratios of approximately 20. Additionally, contributor #17 was evaluated in the H_1 hypothesis and the log likelihood ratios were approximately 20. When the H_1 included just one of the two contributors, contributor #15, the log likelihood jumped to approximately 30. Lastly, when the H_1 included both contributors #15 and #17, the log likelihood ratios jumped to nearly 50. These values are represented in the figure below.

Figure 7:



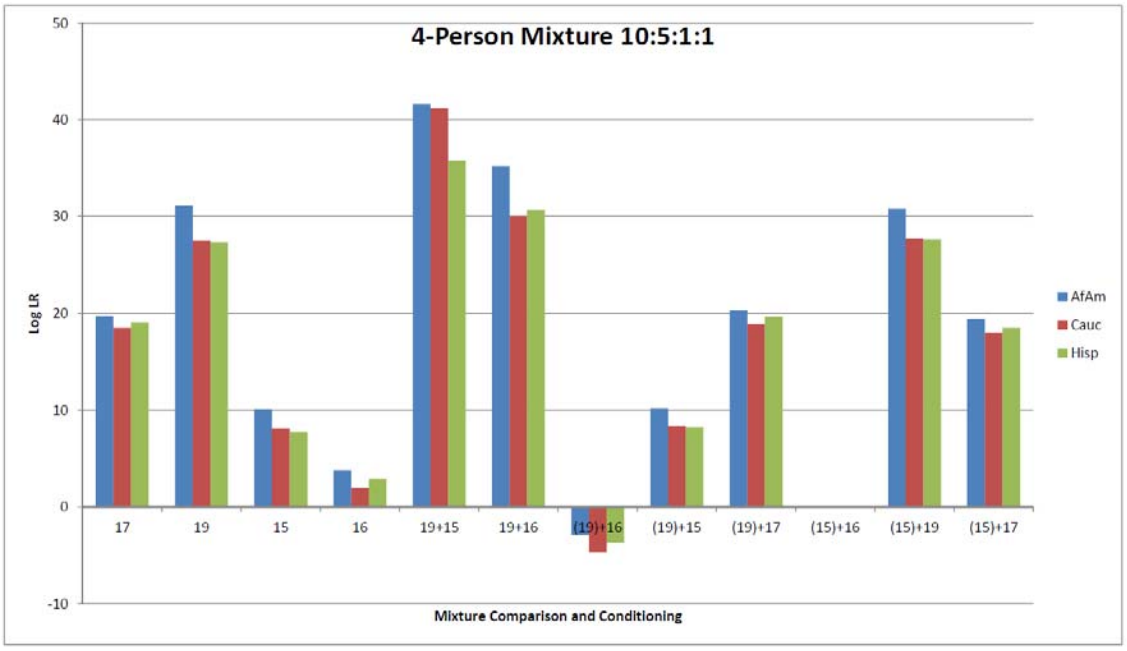
The 3-person mixture was a 10:5:1 mixture of each contributor. This ratio represents a common scenario of having a major, mid and minor contributor. Once deconvoluted, likelihood ratios were calculated for conditioning on the major, mid and minor contributors independently or combined. For this particular mixture, contributor #15 was evaluated in the H_1 hypothesis. This scenario provided log likelihood ratios of approximately 22. Additionally, contributor #16 was evaluated in the H_1 hypothesis and the log likelihood ratios were approximately 18. Finally, contributor #19 was evaluated in the H_1 hypothesis and the log likelihood ratios were approximately 13. These represented each contributor individually evaluated. When the H_1 included just one of the two contributors for conditioning, contributor #19, the log likelihood jumped to approximately 25 for contributor #15 and 20 for contributor #16. Additionally, when contributor #19 was conditioned and contributors #15 and #16 were evaluated jointly the log likelihood ratios jumped to nearly 50. Other combinations of two contributors in the H_1 moved the log likelihood ratios to more than 30. Lastly, when the H_1 included all three contributors, #15, #16 and #19, the log likelihood ratios jumped to nearly 60. These values are represented in the figure below.

Figure 8:



The 4-person mixture was a 10:5:1:1 mixture of each contributor. This ratio represents a common scenario of having a major, mid and multiple minor contributors. Once deconvoluted, likelihood ratios were calculated for conditioning on the major, mid and minor contributors independently or combined. For this particular mixture, log likelihood ratios for each individual contributor ranged from approximately 2 to nearly 30. This range is based on the deconvolution of the 4-person mixture and the amount each individual contributed. Combinations of contributors in the H_1 were evaluated in had significant bearing on the overall log likelihood ratios that were generated. Conditioning on a single contributor #19, improved to various extents the log likelihood ratios calculated. For contributor #16 conditioning had little impact on the very low log likelihood ratios. This is likely due to the overall makeup of the mixture and the various proportions of each person to the mixture. The various scenarios and log likelihood values are represented in the figure below.

Figure 9:



4.1.3 Variable DNA typing conditions

The MSP Biology/DNA unit procedure manual for Fusion does not allow for variations in the number of cycles during the amplification process. Additionally, the procedures do not allow for any post-amplification enhancements such as desalting or concentrating. Lastly, the capillary electrophoresis instrument injection and run conditions allow for a total of three injections conditions and no other variations in conditions. The injection times include an initial 18 seconds with the options for 10 seconds or 28 seconds for improvements to the quality of the electropherograms. The internal validation of STRMix™ included data from 10, 18 and 28 second injections.

4.1.4 Allelic peak height, to include off-scale data

This study was not conducted. The Model Maker analysis indicated that our saturation level of the 3500 Genetic Analyzer detector was at 31,000 RFUs. Allelic peaks less than 31,000 RFUs are within the linear range whereby the stutter percentages can be reliably determined and evaluated within the biological model. Allelic peaks that are greater than 31,000 RFUs would be

outside of the linear range whereby the stutter percentages cannot be reliably determined because they would not be truly representative of the actual stutter percentage value.

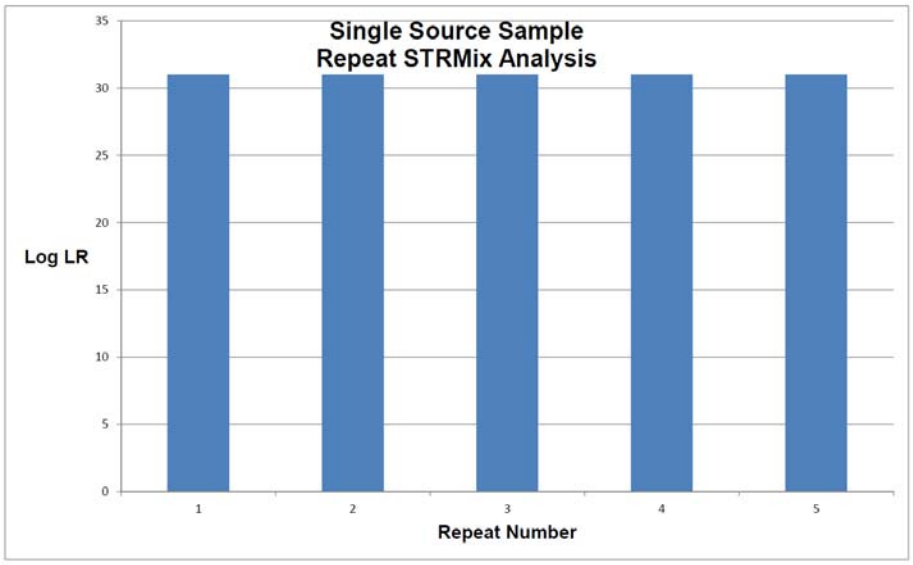
Currently, we do not have an upper threshold for RFU levels, but it is our experience that amplification of samples resulting in RFUs near 31,000 often result in significant amounts of artifacts including spectral pull-up, increased stutter ratios and baseline artifacts. Due to these concerns, our procedure will include a requirement to improve the quality of the electropherograms before conducting STRMix™ analysis where the data include allelic peaks with values greater than 25,000 RFUs. If allelic peaks are greater than 25,000 RFUs after attempts to reduce them through either re-amplification or adjustments to the genetic analyzer, the genetic markers that exceed the 25,000 RFUs may be excluded from the STRMix™ analysis. If extenuating circumstances require inclusion of allelic peaks greater than 25,000 RFUs but below the 31,000 RFU range, supervisor approval may be required.

4.1.5 Single-source specimens

A single source DNA sample prepared from extracted DNA and the concentration determined through the use of Plexor® HY and an Applied Biosystems 7500 was used in two single source studies.

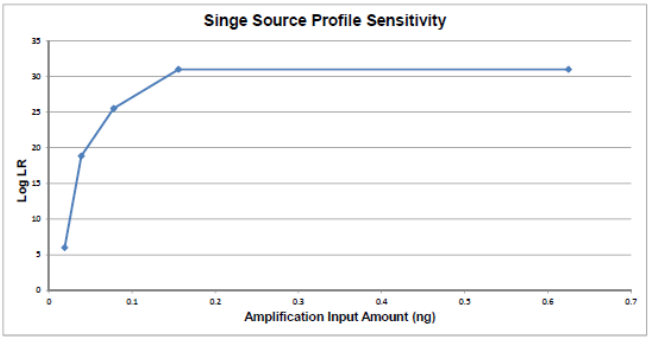
The first study utilized a single electropherogram from this single source sample amplification and analyzed a total of 5 times in STRMix™. The replicate analysis showed that STRMix™ consistently provides the same likelihood ratio for single source samples.

Figure 10:



The second study using a single source sample was termed a sensitivity study. The sensitivity study looked at the serial dilution of the single source DNA extract prior to amplification using standard procedures. The intent of this study was to force STRMix™ to consider the possibility of dropout at both the allele and locus levels and to assess the impact on the likelihood ratios when this occurs. The log likelihood was plotted against the input DNA amount to further depict the impact of dropout. This plot is depicted in Figure 7.

Figure 11:

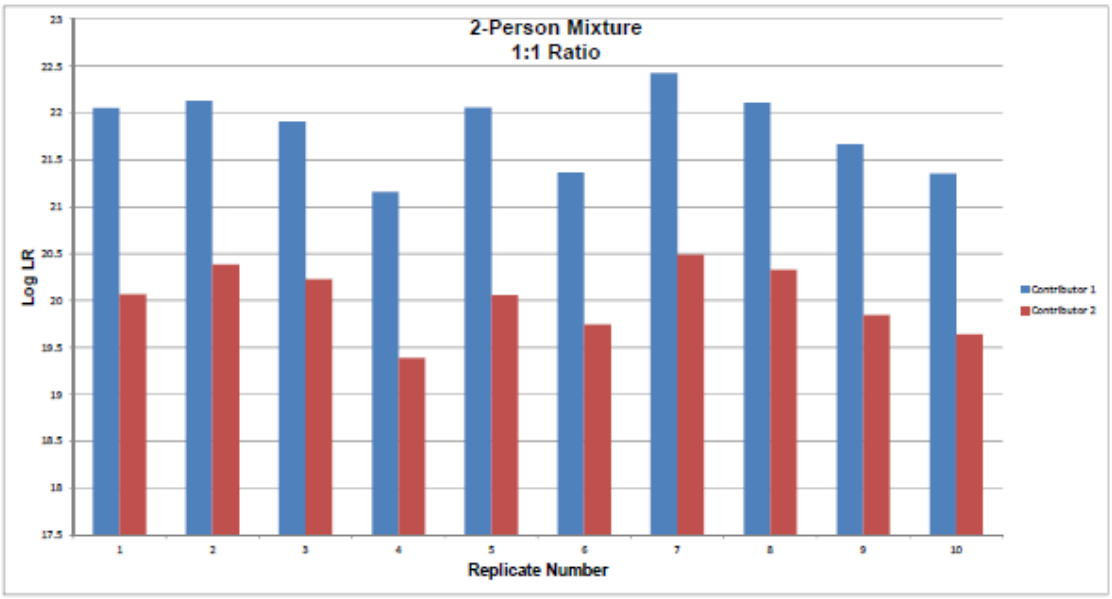


It should be noted that STRMix™ appropriately considers dropout at both the allele and locus levels. This is evidenced by an overall reduction in the likelihood ratio values as the input amplification amount is decreased. As dropout is considered, the potential for the dropped allele to be any known allele decreases the overall likelihood ratio of the profile.

4.1.6 Mixed Specimens

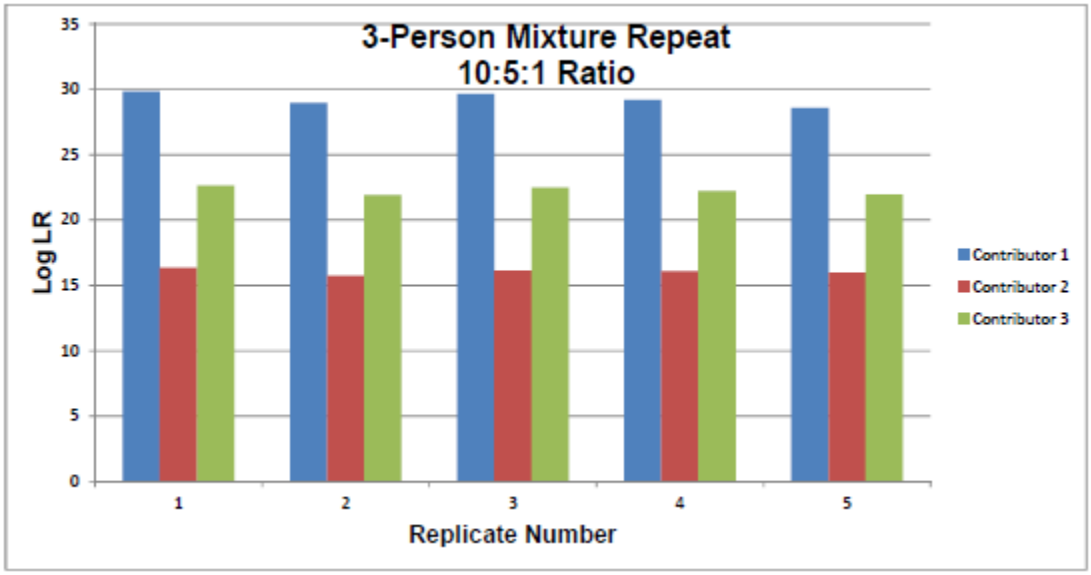
A study was conducted using mixed DNA profiles to determine the repeatability of the STRMix™ analysis. A 2-person DNA profile was prepared in the laboratory at a ratio of 1:1. The 1:1 mixture represents one of the more challenging mixtures to deconvolute in the 2-person category. The STRMix™ analysis was repeated ten times and the log likelihood ratios were calculated for each contributor and each analysis. Figure 12 represents the results obtained.

Figure 12:



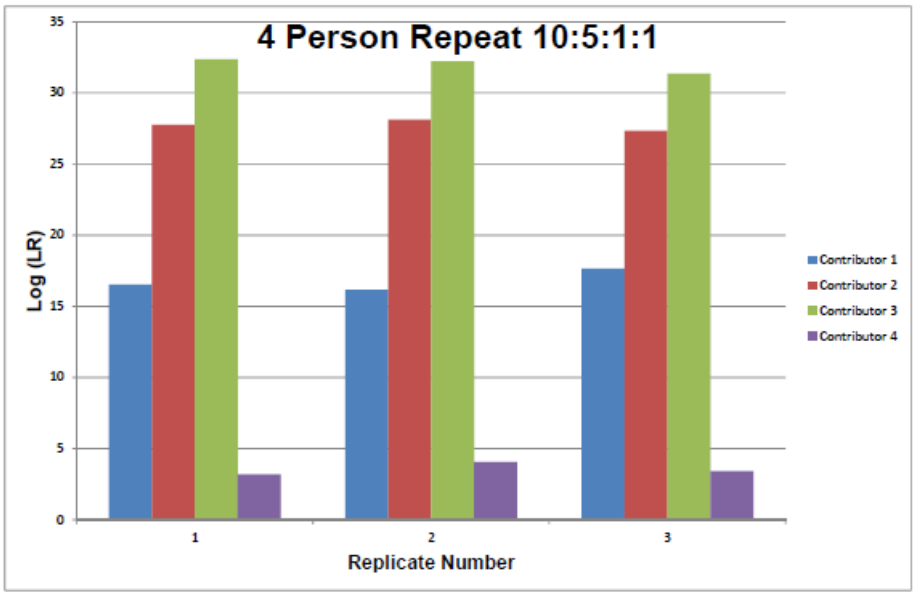
A 3-person DNA profile was prepared in the laboratory at a ratio of 10:5:1. The STRMix™ analysis was repeated five times and the log likelihood ratios were calculated for each contributor and each analysis. Figure 13 represents the results obtained.

Figure 13:



A 4-person DNA profile was prepared in the laboratory at a ratio of 10:5:1:1. The STRMix™ analysis was repeated three times and the log likelihood ratios were calculated for each contributor and each analysis. Figure 14 represents the results obtained.

Figure 14:



It should be noted that in all cases (2-, 3- and 4-person mixtures), the log likelihood ratios generally varied by not more than one order of magnitude.

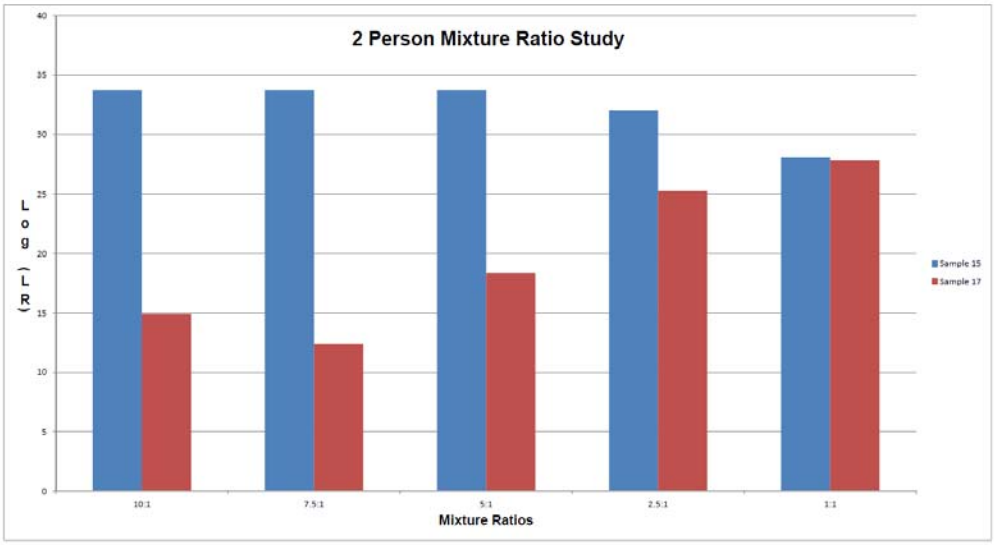
4.1.6.1 Various contributor ratios

DNA reference samples were extracted using standard organic extraction methods and purified using Vivacon® centrifugal collectors. The concentration of the DNA samples was evaluated using Plexor® HY quantitation chemistry and an Applied Biosystems 7500 Real Time PCR instrument. DNA mixtures were prepared in the laboratory from the DNA reference samples. The mixtures covered the range of contributors anticipated in casework including 2-, 3- and 4-person mixtures. The mixtures also included a range of contributor ratios anticipated in casework. The 2-person mixtures included ratios of 10:1, 7.5:1, 5:1, 2.5:1 and 1:1. The 3-person mixtures included ratios of 10:1:1, 10:2:1, 10:5:1, 10:10:1, 10:10:2, 10:10:5 and 10:10:10. The 4-person mixtures included ratios of 10:1:1:1, 10:5:1:1 and 10:10:5:1. Once prepared, the DNA extracts for these mixtures were amplified using standard MSP procedures including the PowerPlex® Fusion STR amplification chemistry at 30 cycles and separated and detected using the Applied Biosystems 3500 Genetic Analyzer. Resulting electropherograms were evaluated using GeneMapper® IDx and exported to STRMix™ via the use of a .txt table file.

Each mixture was evaluated in STRMix™ and compared to known contributors of the mixtures (ground truth) as well as 320 DNA profiles from randomly selected individuals known not to have contributed to the mixtures.

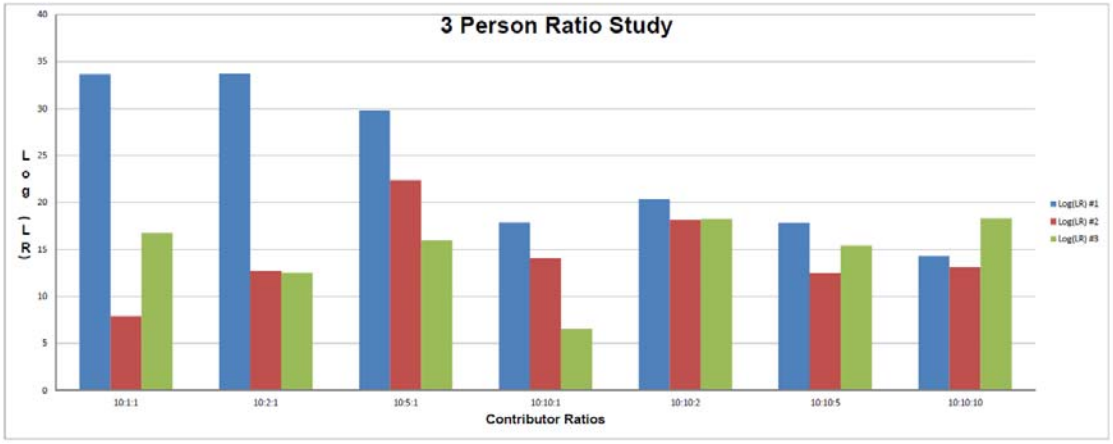
The 2-person mixtures resulted in LRs for the known contributors that were very significant and LRs of zero for those individuals known not to have contributed to the mixtures. The data are represented in Figure 15.

Figure 15:



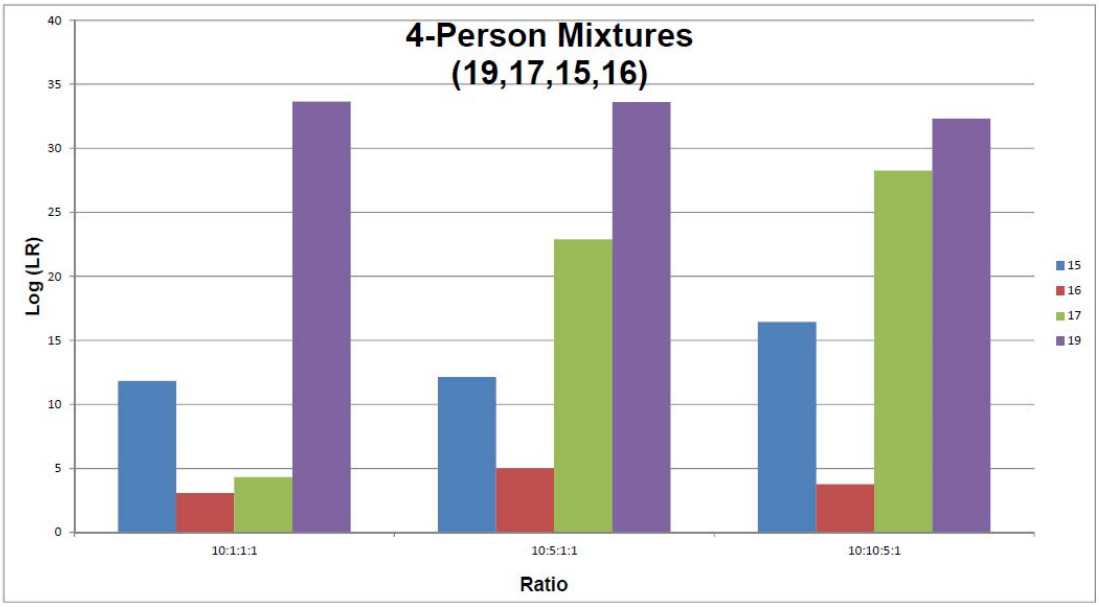
The 3-person mixtures resulted in LRs for the known contributors that were very significant and LRs of zero for those individuals known not to have contributed to the mixtures. The data are represented in Figure 16.

Figure 16:



The 4-person mixtures resulted in LRs for the known contributors that were very significant and LRs of zero for those individuals known not to have contributed to the mixtures. The data are represented in Figure 17.

Figure 17:



4.1.6.2 Various total DNA template quantities

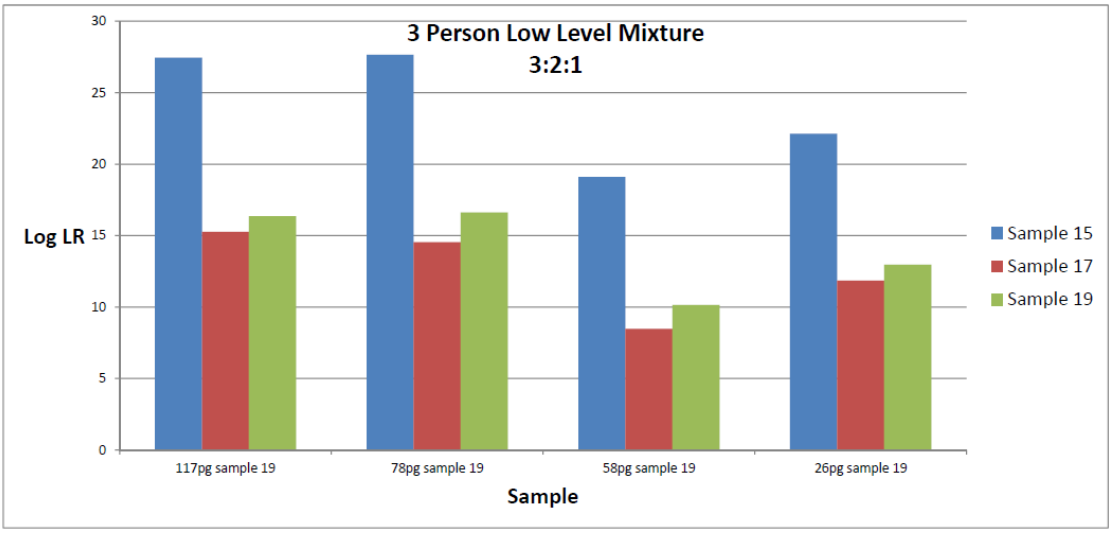
The input DNA amount into the PowerPlex® Fusion amplification reaction can have a dramatic impact on the quantity and quality of the STR results obtained. Generally, the MSP laboratories target between 0.5 and 1.0 ng of input DNA. However, when considering mixtures, the ratio of the mixture and the relative amount of DNA going into the STR reaction must be considered. For instance, a 10:1 two-person mixture that targets 1.0 ng will effectively have approximately 0.1ng of input DNA for the minor contributor.

To address the issue of input amounts for various numbers of contributors, a study was prepared to look at 2-, 3- and 4-person mixtures. A single contributor ratio for each number of contributors was evaluated with this study.

For the 2-person study, a 2.5:1 mixture was created and the input amount was varied. 1µL of the mixture, representing a total input DNA amount of 1ng was evaluated. The major contributor had a likelihood ratio of 3.9E+32 and the minor had a likelihood ratio of 4.7E+25. The same mixture was amplified at a total input DNA amount of 3ng. It was evaluated using STRMix™. The major contributor had a likelihood ratio of 3.6E+33 and the minor had a likelihood ratio of 4.3E+30. Regardless of template amount, a significant likelihood ratio was obtained.

A 3-person mixture study was conducted to evaluate the effects of input amounts on the resulting STRMix™ analysis. A 3:2:1 mixture was created in the laboratory and input DNA amounts for PowerPlex® Fusion were targeted in relation to the minor contributor. The input amounts included 117pg, 78pg, 58pg, 26pg. In these ranges, the minor contributor was either fully represented or some level of allelic and/or locus drop-out was observed. Figure 18 represents the results:

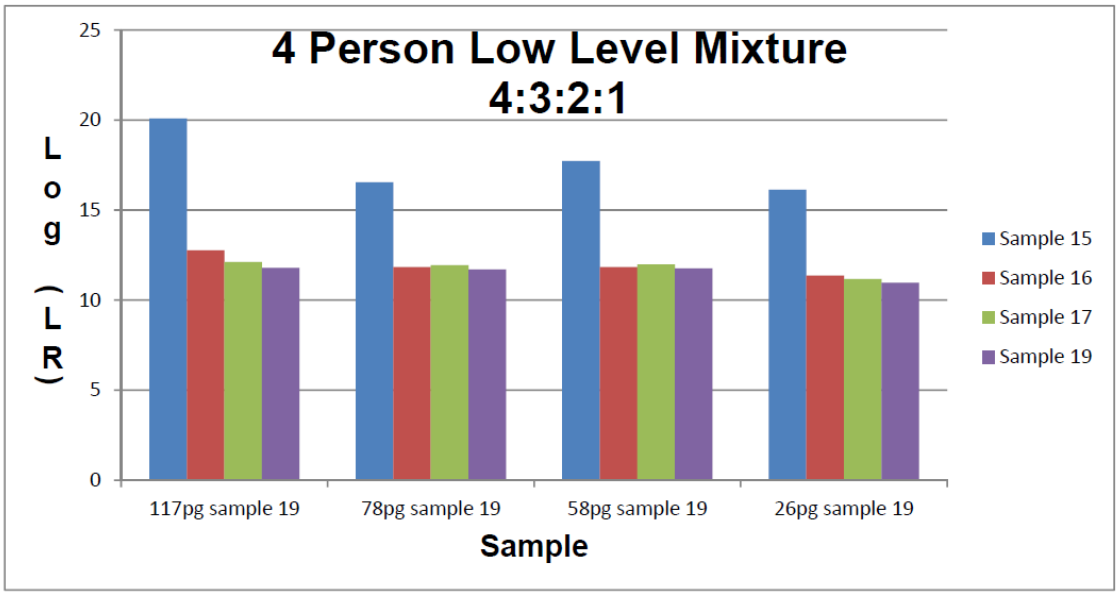
Figure 18:



It can be noted that under all circumstances, significant likelihood ratios can be obtained with the use of STRMix™ at all input DNA amounts tested. However, the significance of the likelihood ratios are negatively impacted as the input DNA amount decreases and the extent of allelic and locus drop-out increases.

A 4-person mixture study was conducted to evaluate the effects of input amounts on the resulting STRMix™ analysis. A 4:3:2:1 mixture was created in the laboratory and input DNA amounts for PowerPlex® Fusion were targeted in relation to the minor contributor. The input amounts included 117pg, 78pg, 58pg, 26pg. In these ranges, the minor contributor was either fully represented or some level of allelic and/or locus drop-out was observed. Figure 19 represents the results:

Figure 19:



As was seen in the 3-person study, the 4-person study also showed significant likelihood ratios obtained regardless of the input DNA amounts. However, there were slight decreases noted as the input DNA amount was decreased.

4.1.6.3 Various numbers of contributors

The Michigan State Police does not anticipate using STRMix™ for DNA profiles that contain greater than four contributors at this time. For this reason, the validation looked at single, two-person, three-person and four-person mixtures only.

A single source sample represents very little modeling required within the STRMix™ application to properly interpret it. Because of this, only one single source sample was utilized during the internal validation. The single source sample was amplified at a variety of input DNA quantities as well as analyzed within STRMix™ repeatedly over a single DNA input quantity. These sets of data were previously described under the single source sensitivity and single source repeatability sections.

For 2-person mixtures, three different reference DNA extracts were utilized. They were sample 15, 17 and 19. They were prepared in the laboratory to represent different ratios commonly encountered in casework. These mixtures included:

- 10 (sample 15) to 1 (sample 17)
- 7.5 (sample 15) to 1 (sample 17)
- 5 (sample 15) to 1 (sample 17)
- 2.5 (sample 15) to 1 (sample 17)
- 1 (sample 15) to 1 (sample 17)
- 10 (sample 15) to 1 (sample 19)
- 7.5 (sample 15) to 1 (sample 19)
- 5 (sample 15) to 1 (sample 19)
- 2.5 (sample 15) to 1 (sample 19)
- 1 (sample 15) to 1 (sample 19).

Not only were these ratios analyzed within STRMix™, but different overall input quantities were evaluated as well. The input quantities were not low enough to generate profiles with drop out at the allelic or locus levels, however. Under all ratios and input DNA quantities, STRMix™ was able to analyze the data and produce very significant likelihood ratios when compared to the actual DNA profile contributors.

For 3-person mixtures, three different reference DNA extracts were utilized. They were sample 15, 16 and 19. They were prepared in the laboratory to represent different ratios commonly encountered in casework. These mixtures included:

- 10 (sample 15) to 1 (sample 16) to 1 (sample 19)
- 10 (sample 15) to 2.5 (sample 16) to 1 (sample 19)
- 10 (sample 15) to 5 (sample 16) to 1 (sample 19)
- 10 (sample 15) to 7.5 (sample 16) to 1 (sample 19)
- 10 (sample 15) to 10 (sample 16) to 1 (sample 19)
- 10 (sample 15) to 10 (sample 16) to 2.5 (sample 19)
- 10 (sample 15) to 10 (sample 16) to 5 (sample 19)
- 10 (sample 15) to 10 (sample 16) to 7.5 (sample 19)
- 10 (sample 15) to 10 (sample 16) to 10 (sample 19).

Not only were these ratios analyzed within STRMix™, but different overall input quantities were evaluated as well. The input quantities were not low enough to generate profiles with drop out at the allelic or locus levels, however. Under all ratios and input DNA quantities, STRMix™ was

able to analyze the data and produce very significant likelihood ratios when compared to the actual DNA profile contributors.

For 4-person mixtures, four different reference DNA extracts were utilized. They were sample 15, 16, 17 and 19. They were prepared in the laboratory to represent different ratios commonly encountered in casework. These mixtures included:

- 10 (sample 19) to 1 (sample 1176) to 1 (sample 15) to 1 (sample 16)
- 10 (sample 19) to 1 (sample 1176) to 1 (sample 15) to 1 (sample 16)
- 10 (sample 19) to 2 (sample 1176) to 1 (sample 15) to 1 (sample 16)
- 10 (sample 19) to 5 (sample 1176) to 1 (sample 15) to 1 (sample 16)
- 10 (sample 19) to 10 (sample 1176) to 1 (sample 15) to 1 (sample 16)
- 10 (sample 19) to 10 (sample 1176) to 2 (sample 15) to 1 (sample 16)
- 10 (sample 19) to 10 (sample 1176) to 5 (sample 15) to 1 (sample 16)
- 10 (sample 19) to 10 (sample 1176) to 10 (sample 15) to 1 (sample 16)
- 10 (sample 19) to 10 (sample 1176) to 10 (sample 15) to 2 (sample 16)
- 10 (sample 19) to 10 (sample 1176) to 10 (sample 15) to 5 (sample 16)
- 10 (sample 19) to 10 (sample 1176) to 10 (sample 15) to 10 (sample 16)

Not only were these ratios analyzed within STRMix™, but different overall input quantities were evaluated as well. The input quantities were not low enough to generate profiles with drop out at the allelic or locus levels, however. Under all ratios and input DNA quantities, STRMix™ was able to analyze the data and produce very significant likelihood ratios when compared to the actual DNA profile contributors. However, in many instances a search against a random database of true non-contributors produced likelihood ratios less than but approaching 1.

See section 4.1.6.2 for the results of variations in the input DNA amounts on the overall STRMix™ analysis results.

4.1.6.4 If the number of contributors is input by the analyst, both correct and incorrect values should be tested.

STRMix™ requires the user to evaluate the mixed contributor profile and consider the number of donors in the mixture. The MSP procedures will allow for single source, 2-person, 3-person and 4-person mixtures to be interpreted using STRMix™.

2-Person mixtures were evaluated using STRMix™ as a single sourced profile. However, STRMix™ produces an error indicating the mixed profile cannot be explained as a single source sample. The same 2-person mixture was evaluated using STRMix™ as a 3-person mixture. STRMix™ will successfully consider this number of contributors. Generally, it will provide you contributor genotypes for the first two contributors accurately, but the third contributor can be many possible combinations of the alleles. A 2-person contributor mixed profile was not evaluated at the 4-person level because users will generally not consider 4 contributors in these situations.

3-person mixtures were evaluated as 2-person and 4-person mixtures. Again, the 2-person option yielded an error within STRMix™ due to the profile not being able to be explained. The same 3-person profile was evaluated as a 4-person mixture. STRMix™ evaluated the mixture and provided significant likelihood ratios for the true contributors of zero or less than one for true non-contributors.

4-person mixtures were not evaluated with the incorrect number of contributors because studies have already shown that STRMix™ will provide an error if the number of contributors is underestimated and we are not validating 5-person mixtures.

4.1.6.5 Sharing of alleles among contributors

A study specific to allele sharing was not conducted in this internal validation. Most, or nearly all, of the mixtures in the internal validation had some level of sharing among the various contributors to the mixed profile.

4.1.7 Partial profiles, to include the following:

4.1.7.1 Allele and locus drop-out

DNA profiles that covered the range of contributor numbers (single source, 2-, 3-, and 4-person) were evaluated for the impact of drop-out on the STRMix™ analysis. For each category, a single profile was selected and ran without the drop-out occurring and again when the drop-out was created through *in silica* means. The drop-out included approximately three alleles at different genetic markers for each sample analyzed. Table 5 represents the likelihood ratios for the true contributors.

Table 5: Drop-Out Results

Sample Type	Likelihood Ratio (no drop-out)	Likelihood Ratio (drop-out)
Single Source	5.2e33	7.1e30
2-Person	5.2e33 4.7e16	5.12e33 1.2e11
3-Person	4.6e33 3.7e13 8.2e13	4.6e33 5.1e12 8.6e14
4-Person	4.7e11 7.4e22 3.8e33 55000	2.5e12 7.6e22 3.9e33 120000

In all instances, except the 4-person mixture, the likelihood ratios decreased as the drop-out was incorporated. The 4-person mixture profile with the drop-out actually increased in the likelihood ratio, but this is likely the result of the standard order of magnitude difference seen in the STRMix™ analysis variation.

4.1.7.2 DNA degradation

DNA degradation is a common occurrence in casework-type samples. It occurs from biological sources (blood, semen, saliva etc.) being exposed to the elements (sun, heat, humidity, etc.) and/or biological influences (bacterial degradation). A 2-person mixture at a ratio of 60:40 was analyzed using STRMix™ under standard analysis parameters. The same 2-person mixture was modified in silica to force the “major” contributor of the mixture to cross over into becoming the “minor” contributor approximately half way through the electropherogram. Additionally, a 3-person mixture at a ratio of 10:5:1 was analyzed using STRMix™ at standard conditions and again with the “mid” contributor becoming the “minor” contributor approximately half way through the electropherogram. The likelihood ratios were evaluated for each contributor and are depicted in Table 6:

Table 6: DNA Degradation Results

Sample Type	Likelihood Ratio (no degradation)	Likelihood Ratio (degradation)
2-Person	2.78e21 1.99e22	2.41e18 7.19e18
3-Person	3.39e14 5.45e20 4.17e13	3.05e14 5.33e21 2.28e11

As can be seen by the data represented in Table 6, even under significant degradation, STRMixTM was able to assign a significant likelihood ratio to each contributor. However, the likelihood ratios were impacted negatively by the presence of degradation.

4.1.7.3 Inhibition

With the use of PowerPlex® Fusion, inhibition is not nearly as significant as previous chemistries used by the Michigan State Police such as Profiler Plus, COfiler and even to some extent PowerPlex® 16HS. For this reason, a study specific to inhibition was not conducted. However, many of the adjudicated cases analyzed using STRMix™ likely contained some level of inhibition and the results of that study indicated significant likelihood ratios were obtained.

4.1.8 Allele drop-in

4.1.9 Forward and reverse stutter

Allelic drop-in and stutter studies were conducted jointly since these artifacts result in nearly identical scenarios. Drop-in and stutter result in an additional DNA type(s) that must be considered in the STRMix™ analysis. The main difference between stutter and drop-in may be the position and height of these artifactual peaks.

A single source sample was utilized for this study and analyzed using STRMix™ at standard conditions. The same single source sample was modified in silica to create elevated stutter peaks and analyzed a second time in STRMix™. It was determined that an additional peak that must be considered within STRMix™ for a single source sample creates a situation where the user must choose between a single source sample and that of a mixed contributor analysis. If the user selects a single source sample within STRMix™, it significantly changes the STRMix™

analysis for the locus holding the elevated stutter or drop-in allele. For instance, if an additional allele is created at a locus where the true contributor is homozygous, STRMix™ will simply consider the locus to be heterozygous, regardless of the peak height ratios between the two peaks. STRMix™ analysis will likely result in a mismatch and an exclusion of the true contributor. If the additional allelic peak represented by drop-in or elevated stutter appears at a locus where the true contributor is heterozygous, and the user opts to interpret it within STRMix™ as a single sourced sample, the application will provide an error indicating the profile cannot be supported as a single source sample. The user's best option when encountering the potential of allelic drop-in or elevated stutter is to run the sample as both a single source sample and as a 2-person mixed sample. The STRMix™ output can then be compared and evaluated for appropriateness.

In addition to the single source sample study, elevated stutter/drop-in was evaluated in a similar manner using a 2-person and 3-person profile. Table 7 depicts the results.

Table 7:

Sample Type	Likelihood Ratio (no drop-in/elevated stutter)	Likelihood Ratio (drop-in/elevated stutter)
Single Source (as 1 contributor)		0
Single Source (as 2 contributors)	9.97e30	9.96e30
2-Person (as 2 contributors)	2.96e24 3.09e23	1.50e24 1.39e23
2 person (as 3 contributors)		7.85e23 3.50e22
3 person (as 3 contributors)	4.37e28 4.30e17 5.96e21	1.58e29 9.99e8 7.11e21

It should be noted that in many situations STRMix™ is able to model elevated stutter in drop-in to the point of having very little impact on the overall likelihood ratios of known contributors. However, as the number of contributors increases, the negative impact on the likelihood ratio of known contributors does become more substantial.

Conclusions:

The internal validation of version 2.3.07 of STRMix™ represents a body of work that follows the validation guidelines for probabilistic genotyping as published by the Scientific Working Group on DNA Analysis Methods (SWGDAM) and the FBI's Quality Assurance Standards for Forensic DNA Testing Laboratories. The validation covered various scenarios, mixture contributor numbers, mixture contributor ratios and implementation situations that need to be addressed in policy. STRMix™ was found to be a valuable tool in the interpretation of single source and mixed contributor DNA profiles. STRMix™ is another beneficial tool to be used by qualified DNA analysts in the overall DNA interpretation process.

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